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THE NEUTROPHIL IN PERNICIOUS ANEMIA*

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There is comparatively little in American medical literature on the neutrophilic features of pernicious anemia, and the aid which such features can furnish in diagnosis. DeCosta,[8] and Cabot,[5] stated that in many cases the neutrophilic leukocytes do not manifest abnormalities in size and staining reaction, frequently being much smaller than normal. Arneth,[1] published his first paper dealing with variations in the number of lobes of the nuclei of the polymorphonuclear neutrophils in health and disease, but it was not until 1907 that he[2] discovered in two cases of uncomplicated pernicious anemia that there was a definite and marked shift to the right in the neutrophilic leukocytes. Decastello,[9] noted the abnormal number of lobes in the neutrophil nuclei in a case of pernicious anemia after splenectomy, and Brosämlen,[3] noted that there was a shift to the right in cases of pernicious anemia during remission. In the course of a study of the Arneth count, Briggs,[4] was the first in this country to find that in ten of twelve cases of pernicious anemia there was a decided increase in the number of lobes in the nuclei of neutrophils. He also felt that there was no relationship between the severity of the anemia and the degree of the shift.

Naegeli,[11] in a paper on early diagnosis of pernicious anemia was able to demonstrate marked segmentation of the neutrophil nuclei. Piney,[13] stated that there is morphologic evidence of defective function of bone marrow in that hyaline leukocytes are always reduced in number and that there are always a varying number of polymorphonuclear leukocytes of large size with hypersegmented nuclei.

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6–9, 1932.

Cooke,[6, 7] reported twenty cases of pernicious anemia studied by a modification of the Arneth count and found a right shift in all of them.

Neuberger,[12] stated that a left shift occurs only in pernicious anemia with complications. He also came to the conclusion that the return of the right shift to normal could not serve as a criterion for a therapeutic result, since this return may occur only a long time after there has been marked improvement, and even after there has been complete remission.

Jedlička and Beranek, presented a report on thirty-six cases in which lobes were counted and believed that a shift to the left is more characteristic of pernicious anemia than a shift to the right. They emphasized the presence of cells in which the nuclear skein is markedly elongated as well as pyknotic in appearance. They term this characteristic "pathological hyperpolymorphism" and place it next to increased segmentation in significance as a diagnostic aid.

Watkins and Berglund,[14] found that the neutrophil of pernicious anemia remained after the blood had returned to normal. They did not determine if this deviation from normal ultimately disappeared, but felt that it was an aid in the diagnosis of pernicious anemia after the blood had become practically normal. Following the introduction of treatment by liver, Fleming[10] studied a case of pernicious anemia in regard to the lobulation of the neutrophil, both before and after treatment with liver as well as in relapse. He showed that under adequate treatment the increased lobulation disappeared, but that as soon as treatment was discontinued, this change again became apparent.

The features of the blood picture in pernicious anemia may be divided into those changes which occur in erythrocytes and those which occur in granular leukocytes. The changes in erythrocytes may be further subdivided into the degenerative and regenerative. The features which have been emphasized in the past are poikilocytosis, macrocytosis with certain of the cells consisting of the large oval type, hyperchromasia, anisocytosis, polychromatophilia, nucleated red cells of the normoblast and megaloblast series, and basophilic stippling. Many observers have em-

phasized particularly the significance of the megaloblast in the diagnosis, and insist on its presence before making a positive morphologic diagnosis. The question of derivation and differentiation of megaloblasts and normoblasts is still controversial.

The outstanding morphologic feature of pernicious anemia is the macrocytosis. This is most striking since it is present in every field in a well-made smear. Changes in the neutrophil are revealed only by more detailed study of the smear, as in cases of leukopenia and lymphocytosis it may be necessary to make the

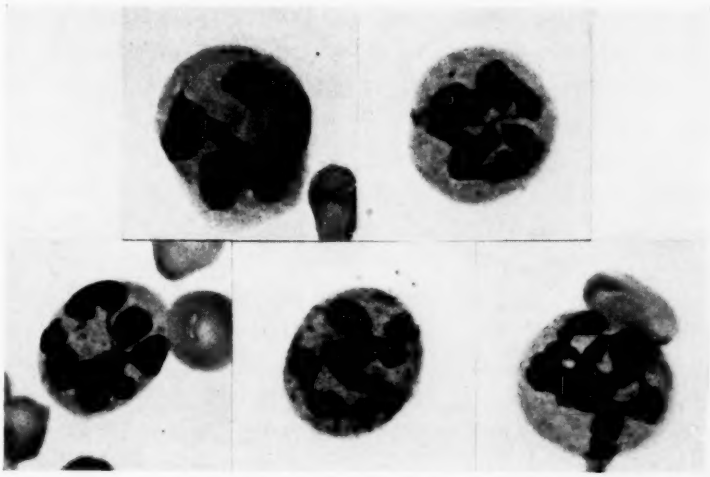

FIG. 1. NEUTROPHILS SHOWING INCREASED SEGMENTATION AND POLYMORPHISM OF THE NUCLEUS

equivalent of a differential count of 300 leukocytes in order to bring out the change.

Since in the observation of patients under treatment all of the foregoing features tend to disappear, and since many early cases may pass unrecognized, one should make use of all diagnostic criteria. We have studied fifty cases in which an unequivocal clinical diagnosis of pernicious anemia was made, in all of which one or more of the morphologic features of the disease were present. All of these patients had achlorhydria. In addition three cases were studied in which there was definite macrocytosis but

in which an adequate quantity of free hydrochloric acid was demonstrated. An average was taken of 100 neutrophils counted by each of us. The criterion laid down by Cooke, that only those lobes should be counted which are definitely separated by thin thread-like strands, has been applied. The difference in criteria of various observers has undoubtedly given rise to the variations in counts of lobes which have been reported. In using the Cooke criterion, neutrophils with marked polymorphism may at first glance appear to be many lobed, but after careful study thread-like filaments will not be seen. The polymorphism as well as increased segmentation is shown in figure 1. Although Schilling recognized the occurrence of a right shift in pernicious anemia, his hemogram in these cases does not actually reveal such a change

TABLE 1

COUNTS OF LOBES IN THREE CASES OF MACROCYTOSIS AND FREE HYDROCHLORIC ACID: DIAGNOSIS INDETERMINATE

NUMBER	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES	LOBES				
				Class 1	Class 2	Class 3	Class 4	Class 5
	gm.	*millions*						
1	9.6	1.96	4,800	8.0	15.0	36.5	24.5	16.0
2	9.7	2.12	6,000	23.5	27.5	30.5	10.5	8.0
3	12.0	2.48	5,500	18.5	25.5	37.5	15.0	3.5

since all cells with a greater number of lobes would be included under the heading "segment forms." Cooke demonstrated that among ninety normal patients the average distribution of lobes was as follows: in class 1, ten cells, in class 2, twenty-five cells, in class 3, forty-seven cells, in class 4, sixteen cells and in class 5, two cells. The occurrence of more than three cells, in class 5, in the normal subject is extremely uncommon. In table 1,* all cells with five or more lobes are included under class 5.

Although for the most part our results agree closely with those of other observers, we cannot confirm the observation that all cases of pernicious anemia manifest increased lobulation or a

* The authors can furnish, upon request, a table of values observed in fifty patients.

right shift in the neutrophils. The usual picture seen is that of macrocytosis with a right shift in the neutrophil. In some of these cases there was a definite tendency to a left shift, although no complicating infection could be found which might give rise to such a change. Our average values for class 1 are considerably higher than those of Cooke. In cases 7 and 28 in which there was no right shift there was definite macrocytosis. On the other hand, two cases, 1 and 49, are included in which there was no macrocytosis but in which there was a definite increased segmentation in the neutrophils. The persistence of macrocytosis, as well as right shift, is demonstrated in case 47 which apparently had been adequately treated. The presence of such changes raises the question of whether the treatment was really adequate, and if it would not be advisable to increase the dosage of active material in an attempt to bring about a normal blood picture. We have seen a few instances in which a positive blood picture persisted for more than a year after institution of treatment with normal blood counts and disappearance of all symptoms. Usually under adequate treatment, the blood picture returns to normal so that no evidence of pernicious anemia can be found. In contrast to the cases mentioned in which the blood picture showed the morphologic changes of pernicious anemia, it is significant that when there is peripheral neuritis of the pernicious anemia type or combined sclerosis with little if any anemia, morphologic examination of the blood smear frequently is not of aid.

Counts of lobes are presented in three cases in which there were morphologic conditions ordinarily associated with pernicious anemia, but in which there was adequate free hydrochloric acid on analysis by test meal. In two of these cases determinations of volume index gave values of 1.13 and 1.45, respectively. In case 2 of this group the response to Lilly's liver extract 343 was excellent. For more than a year the patient had taken an average of twenty-four tubes of this preparation each month without relapse and in the last three months he has remained in excellent condition in spite of the fact that he has not had any treatment directed toward the anemia. In case 1, practically normal blood counts are maintained as long as the patient continues to take

liver extract. One patient (case 3) is under observation at the present time so that data relative to response to treatment are not available. Except for diarrhea in case 2 none of the cardinal symptoms of pernicious anemia was present, and neurologic examination gave entirely negative results.

It is evident from our results that the occurrence of neutrophils with more than five lobes bears no relationship to degree of anemia or to number of leukocytes. It is necessary to emphasize that diagnostic features of the neutrophil in pernicious anemia are not confined to lobulation of the nucleus but that the finer structure, such as elongation of the nuclear skein and connecting strands, thinning of the lobes and slight condensation of chromatin in the lobe, are of significance. In cases in which there is no macrocytosis the finding of right shift or hyperpolymorphism of the neutrophil nucleus may be an aid in the diagnosis.

SUMMARY

Fifty cases of pernicious anemia, in all of which there was achlorhydria, have been studied with reference to the lobulation of the neutrophil. Increased segmentation in the neutrophil is the rule, although cases do occur in which there is a left shift and absence of the hypersegmented neutrophil.

In three cases in which there were morphologic changes of pernicious anemia in the presence of adequate quantities of free hydrochloric acid, but in which other characteristic symptoms of pernicious anemia were absent, no essential differences could be found from the former group.

There is no invariably pathognomonic blood picture of pernicious anemia, since the same changes may be demonstrated in sprue and occasionally in an indeterminate group of anemias.

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SUGAR METABOLISM AND BLOOD STUDIES FOLLOWING VAGOTOMY

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In the course of experiments on the effect of section of the vagus nerve on gastric secretion,[5] it was found that the majority of the animals following vagotomy lost a considerable amount of weight and some succumbed with convulsions. At that time we were unable to account for the deaths of these animals but we did discover that when they were fed with milk instead of the usual laboratory diet they invariably increased in weight and never manifested convulsive seizures. Vanzant[11] had also noticed that some vagotomized dogs lost weight but when fed with fresh meat and milk they regained their normal health.

No adequate explanation has been offered for this phenomenon but it occurred to us that there may have been some factor in the milk which prevented convulsions and loss of weight and which promoted well-being. For this reason it was decided to study the chemistry of the bood in a group of animals in order to determine whether variations occurred in the calcium, phosphorus, sugar, urea nitrogen, carbon-dioxide combining power and chlorides. So far as we have been able to ascertain, no one has satisfactorily studied the complete blood chemistry in vagotomized dogs.

BLOOD CHEMISTRY STUDIES

For this experiment ten dogs were utilized. In eight the right vagus was severed and in two, both vagi were cut just below the diaphragm. Blood chemistry studies were made before this operation was performed in order to establish a normal. The normal varied in different dogs as well as in the same dog and for

that reason the results may be less conclusive than otherwise. After vagotomy, blood for chemical determinations was taken at intervals over a period of four months in some instances. All values were obtained after a twenty-four hour fast.

Table 1 gives the average values in each of the eight vagotomized dogs before and after the operation. In dogs 2 and 4 the

TABLE 1

AVERAGE BLOOD CHEMICAL VALUES*

DOG	SUGAR	UREA NITROGEN	CO_2 COMBINING POWER	CALCIUM	PHOSPHORUS	CHLORIDES
A. Before unilateral vagotomy						
1	92	22.8	47.5	10.5	4.5	493
2	82	40.5	38.7	10.4	5.3	505
3	79	20.8	45.3	10.0	6.3	501
4	72	11.2	52.8	11.3	5.8	504
5	69	14.8	47.5	10.7	3.3	486
6	73	8.9	57.6	10.3	3.0	465
7	82	9.5	48.1	10.4	3.1	536
10	85		52.2	11.6	6.6	556
B. After unilateral vagotomy						
1	30	28.0	21.1	10.6	12.3	592
2	73	17.8	44.6	10.6	5.3	523
3	83	26.7	47.7	9.7	5.0	490
4	76	20.3	48.9	9.6	4.8	502
5	76	13.3	49.9	8.8	4.5	504
6	84	19.5	48.5	9.7	4.7	552
7	died					
10	84	19.6	48.0	10.7	5.0	453

* All values are expressed in mgm. per cent except CO_2 combining power which is in volumes per cent.

urea nitrogen varied over wide limits and in dog 4 there was also a decrease in calcium and phosphorous values after vagotomy. In dog 5 the calcium and phosphorus were lower after vagotomy while the chloride value was somewhat increased. In dog 6 the urea nitrogen values were increased after vagotomy (in dog 2 they were lowered, while in dog 4 they were increased), the calcium

value was lowered while the phosphorous value was increased and the chloride value increased. Dog 1 showed a markedly reduced sugar (hypoglycemic shock), the carbon-dioxide combining power was greatly reduced, the phosphorous value was greatly increased and the chlorides were also somewhat increased. Dog 7 died and dog 3 showed little change before and after vagotomy. Only dogs 1, 4 and 5 had convulsions (see below) in this series. Although there were definite changes in certain of the blood chemical values they were not consistent and whereas in one case the urea nitrogen was lowered after vagotomy, in another it was increased. It would therefore be difficult to draw any definite

TABLE 2

AVERAGE BLOOD CHEMICAL VALUES*

DOG	SUGAR	UREA NITROGEN	CO_2 COMBINING POWER	CALCIUM	PHOSPHORUS	CHLORIDES
Before bilateral vagotomy						
33	—	—	—	—	—	—
9	92	—	54.8	12.0	7.7	515
After bilateral vagotomy						
33	66	13.6	49.5	8.6	4.2	466
9	84	17.6	48.0	10.0	4.6	526

* All values are expressed in mgm. per cent except CO_2 combining power which is in volumes per cent.

conclusions from these results. In the three dogs in whom convulsions occurred number 1 showed hypoglycemia, number 4 an increase in urea nitrogen, and number 5, a decrease in calcium and phosphorous values. Perhaps the most striking of these changes is the extreme hypoglycemic reaction of dog 1; in this dog the blood happened to be taken just at the time of the convulsion which resulted in death. The values obtained in the study of dog 3 represent an example of those usually observed. In this animal there was quite a variation in the urea nitrogen. The sugar values remained within normal limits. The phosphorous values varied between 4.3 and 6.45, the calcium between 8.4 and 10.32

and the chlorides between 453 and 536. These values, however, were considered within normal limits. This dog had no convulsions but did lose weight. All values, of course, are obtained under basal conditions after a twenty-four hour fast.

In two dogs bilateral vagotomy was performed. The effect of this operation upon the blood chemistry is shown in table 2.

In dog 9 there was a reduction in calcium and phosphorus after vagotomy. Unfortunately satisfactory normals were not established in either dog 9 or 33. Nevertheless it is interesting that the normal calcium and phosphorus was so high in dog 9. Both of these dogs lost weight progressively and finally died, dog

TABLE 3

BLOOD CHEMICAL STUDIES* ON DOG 1

DATE	SUGAR	UREA NITROGEN	CO_2 COMBINING POWER	CALCIUM	PHOSPHORUS	CHLORIDES
1/26/32	88		38.5	10.2	4.0	494
1/28/32	110	21.4	44.3	10.4	4.5	524
2/ 2/32	79	24.1	59.8	10.8	5.0	462
2/ 3/32	Vagus section (sub-diaphragmatic)					
2/ 5/32**	30	28.0	21.1	10.6	12.3	592

* All values are expressed in mgm. per cent except CO_2 combining power which is in volumes per cent.

** Died in convulsions, heart's blood obtained immediately. Heart's blood was frequently used during these studies and corresponds closely with values obtained on jugular blood.

33 in nine days, dog 9 in eighteen days. The results of chemical tests done after vagotomy would be considered within normal limits. These results although meagre, would not support a "blood-chemistry" explanation of these deaths.

As has already been recorded, the above experiments were carried out over a period of four months and observations were made at frequent and varying intervals. The first observations were made shortly after vagotomy and these were followed at intervals over a period of four months.

Although convulsions occurred in dogs 1, 4 and 5 all of them lost weight. However, only in dog 1 did death result while the animal was in convulsions. A detailed blood-study on dog 1

revealed the striking hypoglycemia which was not observed in any of the other animals. Table 3 illustrates the blood findings in this animal. Note the remarkably low value of carbon-dioxide combining power and the high value for phosphorus.

LITERATURE

Many years ago, Pavlov[9] came to the conclusion that it was "definitely settled that the vagus is the secretory nerve of the pancreas" and he also remarked

> "how similar the nervous connections of the stomach and pancreas have proved to be; the innervation of the one is in every respect a copy of the other. It is therefore permissible to fill up gaps in our knowledge of the scheme in one case by analogy from the other. We cannot doubt, for example that secretory fibres for the stomach are present not only in the vagus but also in the sympathetic."

According to most anatomists the right vagus nerve sends branches to the pancreas but the left does not. The important fact is, however, that the vagus nerve may by its innervation of the stomach, pancreas, etc. alter, at least temporarily and theoretically the chemistry of the blood or the blood's ability to react to certain chemical changes. If this did occur it might be ascertained by insulin tolerance tests (see below).

Several years ago Clark[2] investigated the influence of the vagus on the islets of Langerhans. He recalled the work of Banting and Gairns which indicated that the secretion of insulin is not necessarily dependent on the nerve supply to the islets of Langerhans but he did not believe it unreasonable to suppose that the nervous system may influence the secretion of insulin. Furthermore, he remarked that the nerve supply to the islets of Langerhans is conveyed, mainly, if not entirely, in the right vagus and that MacLeod and his co-workers had produced hypoglycemia by stimulation of the right vagus in the neck. In Clark's experiments the glucose tolerance test tended to show that sufficient insulin could be produced to meet an emergency without vagal control. In general, Clark's results suggested that stimulation of the vagus caused a secretion of insulin with hypoglycemia. Later he[3] sectioned the right vagus in the neck and found that the

animals (rabbits) went along quite all right for many months showing no change in blood sugar level. "It is clear that the essential function of the islets in the metabolism of sugar is carried on in spite of the nerves having been cut." He was unable to demonstrate decreased sugar tolerance following right vagotomy within a period of two months. On the contrary, in a few cases an increase in tolerance was suggested. He concluded that after vagotomy the sugar tolerance is for some weeks increased and not diminished but that the increased tolerance after vagotomy gradually declines and is later followed by diminished tolerance. In a subsequent paper, Clark[4] concluded that

"section of the right vagus above or below the diaphragm in cats under amytal anesthesia produces an immediate fall in blood sugar, the degree of which varies with (a) the initial blood-sugar level and (b) the glycogen reserve. It is suggested that this effect is produced by cutting off tonic inhibitory influences which control insulin secretion."

MacLeod[7] cautioned that

"since in all these animals (rabbit, cat and dog) a certain degree of variation is observed, even when the conditions of feeding of housing and of preliminary treatment are standardized as far as possible, it is necessary to know, not only the average, but also the extent of deviation."

In normal unanesthetized dogs the blood sugar values vary from 0.057 to 0.088 per cent according to the majority of investigators.

After insulin the picture of hypoglycemia is rather characteristic. These symptoms so closely resembled those shown by our animals dying in convulsions after vagotomy that it is important to describe them here. In the dog

"the first signs are usually very rapid breathing, restlessness and general hypersensitivity, coupled with loss of interest in its surroundings and indifference to the attention of its master. Muscular twitching then becomes evident, and the sphincters may relax. Barking is often a prominent symptom, and there may be salivation and frothing at the mouth. At this stage, or it may be as the first symptom, convulsions, not unlike those seen in the rabbit, may supervene, and between them the animal lies on its side evidently unconscious, showing violent twitchings of the musculature, amounting almost to tetany. The rates of

breathing and of the pulse increase. Inspiration is usually short and jerky and inspiratory tetanus not infrequent, so that artificial respiration may have to be applied. Attempts to get on its feet are often the cause for convulsive seizures, and during recovery the muscles of the extremities, particularly the anterior, are seen to have entirely lost their power of coördinate action."[7]

Although convulsions may be due to acidosis, alkalosis, disturbed calcium-phosphorous ratios, to hypoglycemia or hyperglycemia, gastric tetany (loss of chlorides) et cetera, Hosoi and Alvarez[1] some years ago were convinced, as a result of their work, that both the vagus and splanchnic nerves served largely as brakes to the bowel and that without these brakes the bowel tends to respond to every stimulus and the animals often die of diarrhea and inanition. Our dogs manifested both symptoms but usually seemed to die of the latter and it is possible that this disturbed physiological mechanism may ultimately be found to explain the entire picture. Nevertheless, hypo- and hyper-insulin states have recently attracted so much attention in medicine that this explanation must be given sufficient consideration.

It is interesting that La Barre[6] established an average blood sugar level of 73 mgm. per cent in normal dogs fasting forty hours and found the value to be practically the same after vagotomy above the diaphragm. He also noted that after the administration of glucose there was an earlier return to the original level in the vagotomized animals and that the fall below the original level was more marked than in the normal animals. Later Quigley, Halloran and Barnes[10] reported that chronic vagotomy did not reduce the sensibility of the mechanism which is stimulated by glucose but on the contrary seemed to make it more irritable. For example, they observed that vagotomized animals metabolized "administered glucose" more rapidly than the normal animals.

SUGAR TOLERANCE STUDIES

The record of dog 1, lead us to investigate the sugar metabolism in a series of dogs. The plan was to investigate the response of normal animals to insulin and glucose (glucose tolerance test) and then to examine the same animals after vagotomy. In eight

normal dogs a sugar tolerance test was performed (25 grams of sugar were given intravenously to each dog of approximately equal weight). In five of these the tolerance curve was normal while in the remaining three (dogs 16, 17 and 18) the blood sugar continued to be high (174–250 mgm. per cent) even after two and one-half hours. Six of these dogs in addition to several others were vagotomized and glucose tolerance tests were repeated several times. The same variations which occurred in the normal animals also occurred in the vagotomized ones and one is inclined to believe after studying such results that vagotomy is probably not a factor in these variable results and that they more likely depend upon some underlying disturbed metabolic phenomenon occurring in each animal. Seven animals (dogs 14 (1), 14 (2), 15, 6, 5, 10, 1b) gave essentially normal tests. Dog 17 gave an abnormal response in each of three tests. Dog 16 responded normally in one test, abnormally in two others; dog 11 responded normally in one test, abnormally in another. Another striking observation is the fact that dog 14 (2) had a blood sugar of 200 mgm. per cent before glucose was given and that dog 17 on one occasion had a blood sugar of 84 mgm. per cent as a normal while in two other instances it was too low to read. Similarly dog 11 had a blood sugar of 76 mgm. per cent in one instance and less than 20 mgm. per cent in another. Notwithstanding these variations the dogs appeared to be normal; there were signs neither of hypo- nor hyper-glycemia.

It had previously been noted that dogs may respond differently immediately after vagotomy as compared with those cases in which months have elapsed since vagotomy. This aspect of the problem was studied and though many variations in sugar values were noted, yet, on the whole, the responses were not significantly different. Most of the dogs returned to normal, some did not; recent and old vagotomies seemed to play no important rôle in sugar metabolism. For example, dogs 5, 6, and 10 had been vagotomized three and a half months before the glucose tolerance tests were performed; dogs 16 and 11 one month before, and dogs 16, 17, 15, 14 (1) and 14 (2) were examined one, two and three weeks after right vagotomy. Dogs 5, 6, and 10

responded normally, dog 16 responds normally but dog 11 has a variable response; of the remaining animals, dogs 14 (1), 14 (2), and 15 responded normally and 16 and 17 did not. It is permissible to conclude that vagotomy may have an occasional immediate disturbing effect but this is never permanent according to these experiments. It seems that the body adjusts itself to vagus section and manages to function normally despite the loss of vagus innervation.

INSULIN STUDIES

In five vagotomized dogs and in three unoperated animals, 12 cc. of insulin (U 20) were injected intravenously in order to determine its effect on sugar metabolism. This dosage of insulin was chosen after gradual increases in insulin were given. This amount was found to produce a rather rapid hypoglycemia with its concomitant symptoms. The blood sugar levels varied similarly in all eight of the animals but in general, the vagotomized dogs seemed weaker and more subject to convulsive seizures and pareses. Three of the vagotomized dogs reacted with incoördination and weakness; one had convulsions, one became very quiet after insulin administration and was evidently profoundly paretic, one remained well even though the chemical determinations were identical with those in the other vagotomized dogs. Of the unoperated animals one remained in good condition, one became weak and unsteady and one went into convulsions but recovered quickly upon the intravenous administration of glucose.

AUTOPSY STUDIES

Autopsies were performed to verify vagus nerve section as well as to discover pathological changes. There were no striking gross changes. The bilaterally vagotomized dogs (9 and 33) showed in the first case a hemorrhagic area 2 cm. in diameter on the greater curvature just above the pylorus and in the latter a chronic passive congestion of the liver, lower lobes of both lungs, spleen and kidney and small hemorrhagic areas throughout the stomach. The other dogs showed no remarkable abnormalities.

In each instance, however, specimens for microscopic examination were obtained from the liver, spleen and from three portions of the pancreas and in all of these microscopic examination revealed no significant changes. It is interesting in this connection to mention the work of Mattioli[8] who injected dextrose and epinephrine into dogs and found, on histological examination of the pancreas, an increase in the number and size of the islands of Langerhans. When insulin was administered in addition to the dextrose a mild hyperplasia in the endocrine part of the pancreas was noted. The author concludes that the endocrine tissue of the pancreas is the principal factor in the mechanism of glycoregulation. These experiments are not really comparable to ours since vagus section was not done.

CONCLUSIONS

(1) In vagotomized dogs changes occur in the blood chemistry values but they are so inconsistent that their significance becomes dubious.

(2) The sugar tolerance test yielded variable and similar results in both the normal and vagotomized dogs. Some normal dogs failed to give normal glucose tolerance curves and many vagotomized dogs did respond normally.

(3) Vagotomy is probably not a factor in the production of these inconsistent reactions to glucose administration.

(4) The minor changes following upon vagus section disappeared quickly; the animals, after three months, responded normally in every way. We were unable to demonstrate any constant increased irritability of the mechanism stimulated by glucose administration nor were we able to conclude that there was an increased sugar tolerance test for the first few weeks after vagus section which later is followed by a diminished tolerance. The longer the studies were carried on (eight months in some cases) the more we were convinced that almost any reaction might be obtained.

(5) The blood sugar values following insulin administration were essentially the same in both normal and vagotomized dogs. Even though there were no significant changes in the sugar values,

nevertheless the vagotomized animals were more apt to manifest symptoms in the nature of weakness, nausea, vomiting, pareses, and convulsions.

(6) Autopsy studies did not disclose any noteworthy pathological changes. Vagotomy did not result in any histological abnormalities in the pancreas.

(7) The convulsions and loss of weight in these dogs can not be explained by demonstrable changes in the blood chemistry nor can they be based upon consistent abnormalities in sugar metabolism. It seems that a definite metabolic disturbance is accountable for this phenomenon and that withholding food certainly plays an important rôle in the production of these symptoms. However, other factors as yet undetermined may be concerned in their development and further studies must be made to discover them.

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LEPTOSPIRAL JAUNDICE

A Report of Two Cases

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The wide geographical distribution of rat carriers of *Leptospira icterohemorrhagiae* as determined by Jobling and Eggstein,[5] Otteraaen,[9] Ridlon,[10] Wadsworth et al.,[15] and Langworthy and Moore,[6] makes the report of these human cases of leptospiral jaundice from southern California desirable. Human cases have now been recorded from Canada to Tennessee and from the Atlantic to the Pacific coasts.

Following the work of Inada and his co-workers,[4] authentic cases occurring in North America were reported by Wadsworth et al.,[15] Sailer,[11] McDowell,[7] Bates,[1] Towler and Walker,[14] Cushing,[2] Hayman and Lynch,[3] and Mulholland.[8] The present cases are the ninth and tenth in American literature.

Sellards and Theiler[13] in 1927 advanced evidence to indicate that *Leptospira icterohemorrhagiae* and *Leptospira icteroides* were identical. Sawyer and his co-workers[12] in 1930 presented evidence indicating that neither African nor American yellow fever are caused by leptospiras. The similarity of the clinical picture in leptospiral jaundice and yellow fever with its consequent mistakes in diagnosis, explains the error of Noguchi in ascribing the leptospira of one disease as the etiologic agent of the other.

CASE HISTORIES

1. The patient, a white Canadian war veteran forty-two years of age, without stated occupation, entered the Los Angeles County General Hospital, Nov. 18, 1928. He was so sick and uncoöperative that but a very meager history could be obtained. He had been ill for two weeks with high fever which subsided with the onset of jaundice five days prior to admittance. He was deeply

jaundiced. He had had generalized abdominal pain at times and had vomited frequently. He had not accepted food for several days, was partially demented, and could not seem to talk above a whisper. Subsequent questioning of a sister revealed the fact that just prior to the present illness he had been cleaning up an old deserted carpet factory.

A previous history in the same hospital revealed the information that there was X-ray evidence of a small calculus at the lower pole of the right kidney, and one at the uretero-pelvic junction of the left, with pyonephrosis. His blood Wassermann at that time was negative; the blood chemistry within normal limits. The stone on the left side was removed one year prior to the present illness, since which time he had experienced a bettering of his general health.

Examination revealed him to be well developed and well nourished, acutely ill, and at times tossing about. The sclerae were yellow. Normal pupillary reactions were present. The mouth was dry; the lips cracked; the tongue coated and swollen. No râles were heard in the lungs. The heart rate was rapid: the blood pressure 130/70. The abdomen was somewhat distended and tympanitic though rigidity and tenderness were present. There was a suggestion of a mass in the gall bladder area. The extremities were negative except for a sluggish patellar reflex.

Laboratory findings: Hemoglobin, 80 per cent; leukocytes, 14,250 per cu. mm.; polymorphonuclear neutrophiles 94 per cent; large lymphocytes 5 per cent; transitionals 1 per cent. Blood chemistry: Sugar, 131 mg. per cent; non-protein nitrogen, 162; creatinine, 3.2; uric acid, 7.1 mg. per cent, respectively. Icteric index, 28.5; Van den Bergh test, direct: positive; indirect: 2.5. Urine: coffee colored, alkaline, specific gravity, 1.016, no sugar present, but a trace of albumin. Bile, four plus. The microscope revealed a few hyaline and granular casts, and an occasional red blood cell cast, as well as clumps of leukocytes.

Progress: A diagnosis of "suppurative cholecystitis with cholangitis and hepatitis" was made, and the gall bladder drained under a local anesthetic. The gall bladder was found greatly distended, thinned, and adherent to all surrounding structures. On incision there was free drainage of bile through the gall bladder. Smear from the gall bladder contents showed pus cells and many gram positive cocci. On culture a staphylococcus and *Esch. coli* (B. coli) were isolated.

Following the operation considerable blood drained through the tube inserted into the gall bladder. Some of this clotted and obstructed the tube. His condition became progressively worse. Death occurred four days after admission.

The autopsy was performed five hours after death and prior to embalming.

The lungs showed evidence of purulent bronchitis and bronchopneumonia in the lower lobes. The heart was not materially enlarged, and aside from bile staining of the serous surfaces there was no evidence of pathology.

The liver was slightly enlarged but retained an essentially normal appearance on cut section. The gall bladder was greatly distended and contained clotted blood. The common and cystic ducts were patent. There were no findings to

indicate an obstructive jaundice. The pancreas showed some degeneration. The spleen was rather large but did not show material gross evidence of pathology.

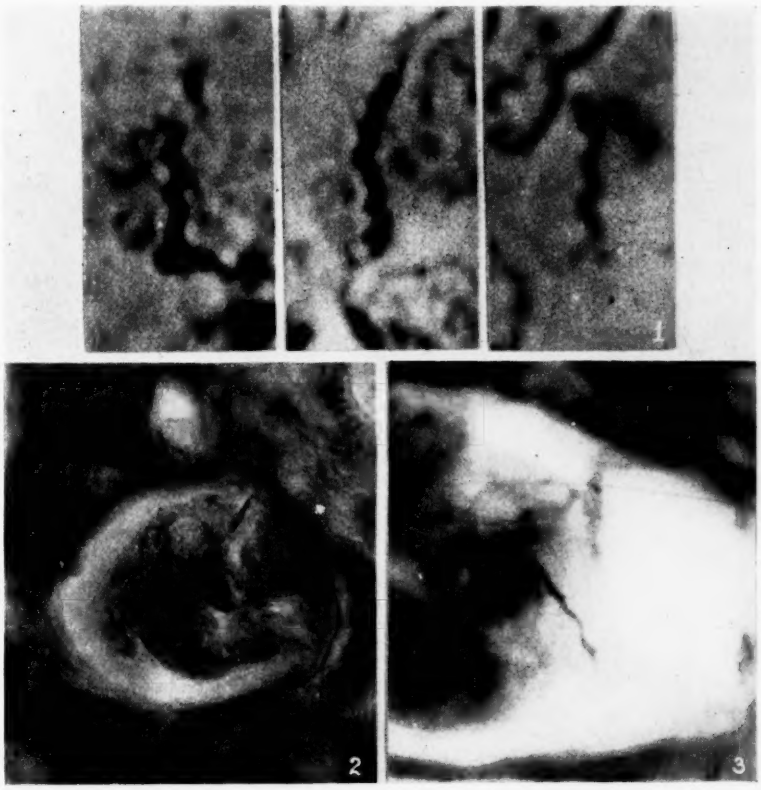

FIG. 1. THREE LEPTOSPIRAS FROM A LEVADITI IMPREGNATION OF THE KIDNEY IN CASE 1
(5500 diameters)

FIG. 2. ORGANISM WITH HOOKED EXTREMITY IN KIDNEY TUBULE OF CASE 2
Levaditi stain (1500 diameters)

FIG. 3. ANOTHER ORGANISM IN A KIDNEY TUBULE
Levaditi stain (3000 diameters)

The left kidney was bound down by adhesions at the site of a previous operation and moderate hydronephrosis was present. There was no evident reason for this hydronephrosis and it may be considered a residuum from the previously

removed calculus. The kidney substance was swollen and hemorrhagic. The right kidney showed rather severe hemorrhages throughout the parenchyma. A hydronephrosis on this side was caused by a small calculus at the ureteropelvic junction.

Rapid frozen sections of liver, pancreas and kidneys revealed petechial hemorrhages in the pancreas, and cloudy swelling and suppuration in the kidneys. The liver showed areas of round cell infiltration and some congestion as evidence of a hepatitis.

Because the jaundice could not be explained on a mechanical basis, and since the microscopic findings were consistent with a generalized infection, the death certificate was signed using "septicemia with hepatitis and jaundice (organism not discovered)," as the cause of death. The possibility of Weil's disease was considered and smears of the kidney made and stained by Fontana's method. These showed definite silver positive wavy forms strongly suggestive of leptospiras, with the one objection that the ends were usually seen to be clubbed or bulbous, although a few forms existed without this appearance. This finding confirmed our suspicion sufficiently so that intraperitoneal inoculation of macerated kidney tissue in saline was made into a guinea-pig. As may be the case, the animal did not develop a definite jaundice, but became somewhat indisposed. It was sacrificed one week after inoculation and the punctate pulmonary hemorrhages characteristic of this infection were demonstrated. From this lung macerated with sterile saline, large numbers of leptospiras with closely wound spirals were demonstrated by dark field technic.

Levaditi's stain on the patient's kidneys revealed definite silver positive forms with tapering ends found sparsely scattered throughout the degenerating kidney substance. These could be satisfactorily classified morphologically as leptospiras (fig. 1). Cultures were not sceured because of the technical difficulties encountered in preparing a satisfactory medium as described by Noguchi, before the viable organisms were lost.

Having established the diagnosis, we were particularly anxious to determine the source of the infection. The relatives persistently refused to reveal the location of the property where the man had been working directly prior to his illness. This was described as an old carpet factory that had not been occupied for some years which was in untidy repair, with newspapers and rags lying about. Careful questioning of these relatives failed to reveal any other clue that might reasonably explain the infection. It is assumed therefore that he may have likely acquired the infection through the abrased or unbroken skin from contact with papers and rags contaminated with urine from infected rats. It is a simple matter

to accept the probable presence of rats in such a place as that described.

2. I. H., a mechanic, fifty-one years of age, entered the San Diego County General Hospital Jan. 16, 1932 presenting a deep jaundice. One month previously, at an unemployment camp, the patient was seized with chills and fever. His left knee became swollen and painful at this time, but subsided in three days. The chills and fever continued for two weeks and subsided just prior to the development of the jaundice. He was not seen by a physician during this time, so that the degree of fever is not known. He was not, however, confined to bed. As the jaundice developed and deepened he was seen by a physician who referred him into the hospital for diagnosis. At this time he complained of the jaundice with itching, black urine, and diarrhea with clay-colored stools. His temperature was normal and remained so until after operation one week later.

Until six months prior to admittance the patient had resided in Central America since 1910 and had malaria with yearly relapses since 1911, black water fever in 1924, and dysentery four times since 1928.

General examination at the time of admittance was negative save for the jaundice and some tympanites with abdominal tenderness. The spleen could not be palpated with certainty. The hemoglobin was 95 per cent (Sahli); erythrocytes, 4,680,000, and leukocytes, 9,000 per cu. mm. with 68 per cent neutrophiles. The Kline and Wassermann tests were negative. Blood sugar was 100 mg. per cent. Icteric index 63; The Van den Bergh test gave a delayed biphasic reaction. Except for bile pigment, the urine showed no abnormality at this time. Three days later, the icteric index was 100. The next day his urine showed a distinct trace of albumin with many hyaline and granular casts and leukocytes. The stool was negative for bile on two occasions five days apart. X-rays of the gall bladder area revealed no calculi.

The patient was prepared for surgery by calcium administration. He was operated upon on January 27, 1932, eleven days after admission, with a diagnosis of obstructive jaundice, likely a calculus. The blood coagulation time was six minutes. No active process was discovered at operation that could account for the jaundice. A cholecystotomy was done and the wound closed in the usual manner. The operation was performed under spinal anesthesia. The post-operative condition was never good. The pulse was weak and thready and very rapid. The patient complained of pain in the lower abdomen and urine obtained by catheter was very bloody. He died on the second post-operative day.

An autopsy was performed eighteen hours after death, subsequent to embalming. The immediate case of death was found to be a large hemorrhage, arising from the lower angle of the wound and extending into the prevesical space which it greatly distended. The blood also infiltrated the bladder at its base, explaining the bloody urine obtained during life.

No extra-hepatic cause for the jaundice could be discovered. The liver was normal in size but presented a marked degree of cloudy swelling and definite areas of focal necrosis observable grossly. The pancreas showed petechial hemorrhages. The spleen was moderately enlarged. The jejunum and upper ileum showed several hemorrhagic areas 2 cm. or more in diameter which were found on the mucosal side to be ulcerated. This undoubtedly had considerable to do with the diarrhea observed clinically. A horseshoe kidney was present which showed distinct degenerative changes of an acute type. Smears from the kidney stained by Fontana's method showed silver positive forms which were thought suggestive of leptospiras. Microscopically, the liver showed many focal areas of complete necrosis, sufficient to explain the clay colored stools on the basis of obstruction to bile excretion by the degenerating liver cells. The kidney also showed similar degeneration. Levaditi impregnation of the kidney revealed occasional silver positive spiral forms, in the degenerating kidney tubules (figs. 2 and 3). These were somewhat thicker in proportion to their length than those of case 1. They also presented fewer secondary spirals. In addition there were found large numbers of silver positive 'ring bodies' and some 'comma' forms suggesting a transition from one phase to the other. These were found in the tubules showing the most extensive degeneration of epithelium. Similar ring forms have been described in syphilitic tissues and are regarded by some[16,17] as a pleomorphic form of the infecting agent.

Since the body had been embalmed prior to autopsy it was not possible to identify the organisms in any other way than by their morphology. A guinea pig was innoculated with a saline suspension of macerated kidney substance but the effort proved futile.

The history of this case is somewhat less complete than that of the first. The early symptoms were much less prostrating, but the progress of the case was of the same general type. Whether the years spent in Central America may in any way explain the decreased severity of the symptoms is problematical. It is even conceivable that a latent infection was acquired in Central America, but not activated until circumstances caused greater exposure. This is perhaps improbable.

Many organisms in the second case differ somewhat from those of the first. They appear more like spirillas than leptospiras. It is possible that the stage of the disease with the evidences of degenerated or involutional forms may account for the apparent decrease in the number of secondary spirals, or the organisms may be actually different types. The clinical symptoms and autopsy findings, however, warrant associating the two cases, at

least until such a time as a clearer differentiation can be made from the study of additional cases.

This second case occurred while the individual was living with an unemployment colony in the barracks of a former army training camp near San Diego. It is not known how the infection was acquired. No other cases have been recognized in the same camp.

A study of the human cases reported in North America makes it appear that this infection has been recognized only in cities of at least moderate size associated with some fairly large body of water. Whether this bears a relation to the incidence of rat carriers is unknown.

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THE LIGHT FILTERING ACTION OF BLOOD SERUM*

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It is a commonplace observation that human blood serums differ in their color and relative opacity to light. While these variations have been studied from the chemical standpoint, so far as I have been able to ascertain, no study has been made of the light filtering action of human blood serum. Our original investigations were based upon photographic procedures which were found to be too complicated for clinical purposes and were therefore discarded. A series of subsequent investigations using the Exton scopometer were also discarded since that instrument was not suited for the type of investigation undertaken. The results recorded in this paper were obtained with the Pulfrich photometer.

METHOD

This photometer consists of a lamp housing, in front of which is a prism which splits the light into two parallel beams of equal intensity. In front of these light beams are placed two standardized cells, which for the purposes of the present investigation have a thickness between the upright walls of 2.5 mm. In the one cell distilled water is placed, while the serum under observation is placed in the other. Next comes an observation tube containing a prism which brings the light beams in contact so that each beam illuminates one half a circle. Between the eyepiece and this prism is a holder in which light filters may be placed. In the present study filters having as their center of filtration the following wave lengths were employed; 7200 Å, 5700 Å, 5000 Å, 4300 Å. In subsequent discussion these filters will be referred to by the first two figures only the zeros being dropped. The filters are so standardized that while they cover a band approximately 100 Å units each side of the designated wave length, their center has, by spectroscopic examination, been determined to be at the point indicated. On each side of the instrument is a wheel which, when rotated, closes a square iris gradually cutting off the light. This wheel is graduated on

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its circumference in percentages, so arranged that the percentage indicated is the amount of light transmitted although the manipulation of the wheel actually cuts off light. In actual use the wheel on the side containing the distilled water is rotated until both fields of light are of equal intensity. The more exact details of the technical procedure are given in the booklet furnished by the manufacturers.

Blood was collected in the usual fashion, by venepuncture, under the standard conditions of procedure as for a blood chemical examination, before breakfast, or after a fast of approximately twelve hours. The serum was rapidly

TABLE 1

LIGHT FILTRATION OF VARIOUS SERUMS

DIAGNOSIS	TYPE A					TYPE B					TYPE C				
	Number of specimens	Per cent	Averaged rates; light filtration for each band			Number of specimens	Per cent	Averaged rates; light filtration for each band			Number of specimens	Per cent	Averaged rates; light filtration for each band		
			72–57	57–50	50–43			72–57	57–50	50–43			72–57	57–50	50–43
Normals	42	40	0.6	2.3	3.3	19	20	1.9	1.4	5.1	43	40	0.5	4.3	2.4
Arterio-sclerosis, hypertension	24	57	0.6	2.4	3.9	5	12	1.5	1.4	5.0	13	31	0.3	3.6	2.4
Mental disturbance	5	28	1.0	2.6	4.2	2	11	1.9	1.4	5.3	10	61	0.6	5.4	4.8
Endocrine dysfunction	8	40	0.5	2.2	3.2	6	30	1.8	1.3	5.6	6	30	1.1	4.0	2.0
Chronic bacterial infection	17	34	0.6	2.0	3.3	5	11	2.4	1.2	5.0	27	55	0.6	3.0	2.0
Acute bacterial infection	33	37	0.6	2.0	3.3	9	10	1.8	1.1	4.9	46	53	0.4	3.0	1.9
Syphilis	20	36	0.5	1.9	3.7	13	24	1.7	1.4	4.9	22	40	0.7	3.2	2.4
Fractures	7	35	0.7	2.1	3.6	4	20	2.0	1.3	5.3	9	45	0.3	3.0	2.9
Pregnancy	10	58	0.6	2.1	3.4	3	19	3.3	1.3	5.6	4	23	0.5	2.3	1.7
Healing wounds	9	50	0.7	2.4	4.2	2	11	4.7	0.8	5.4	7	39	0.9	2.8	2.3
Benign tumors	3	13	0.6	2.0	3.9	9	39	2.3	1.3	4.9	11	48	0.6	3.3	2.9
Malignant tumors	7	30	0.5	1.7	3.9	5	21	1.6	1.0	5.7	11	49	0.5	3.4	1.7

separated from the clot. Hemolized serums were discarded, and the examination was made within twelve hours after the collection of the specimen.

The serum having been separated, one cell was filled with it, the other with distilled water. In the manner indicated in the booklet of instructions, the transmission of light for each of the wave lengths was determined. The rate of absorption of light per 100 Å units for each band between the filters was then determined, as in the following hypothetical example.

Readings; 72 Å, 78 per cent; 57 Å, 70 per cent; 50 Å, 51 per cent; 43 Å, 35 per cent. There are 1500 Å units between 57 and 72, and 700 Å units in each of the other two bands. The band 57 to 72 filtered out 8 per cent of the light

(78 per cent minus 70 per cent). The band 50 to 57 filtered out 19 per cent, and the band 43 to 50 filtered out 16 per cent. The rate in the first band was 8 divided by 15 or one half of one per cent, in the second band 19 divided by 7 or 2.7 per cent and in the third band 16 divided by 7 or 2.2 per cent.

If the three percentages thus obtained are presented in graphic form it will be found that three types of curve occur (see fig. 1). In one termed A, the rate

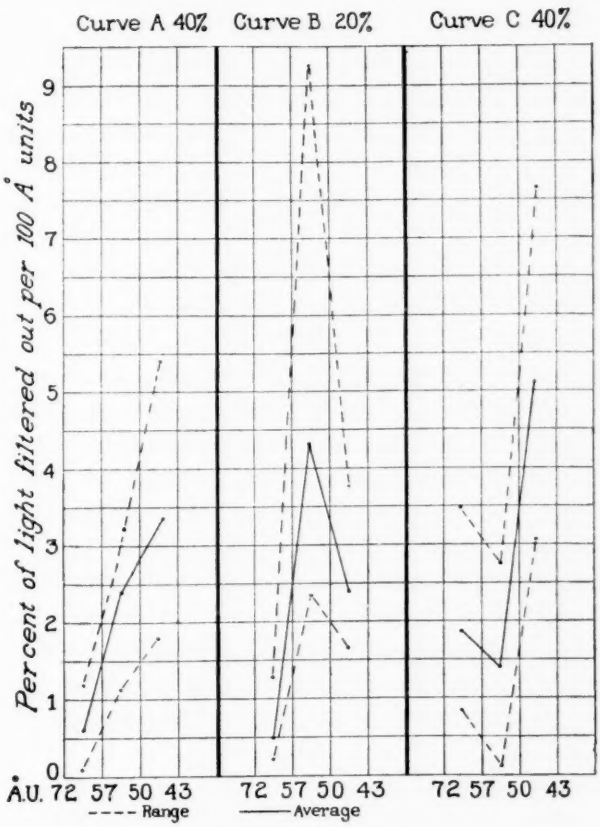

FIG. 1. TYPICAL CURVES

of filtration increases progressively. In the second type termed B, the maximum rate of filtration occurs in the middle band. In the third type, termed C, the minimum rate of filtration occurs in the middle band.

An analysis of 104 normal serums made on the classification just described demonstrates the following: the range of the

reaction, when different serums are compared, is wide (see broken lines fig. 1), the curve types are divided as follows A, 40 per cent, B, 20 per cent, C, 40 per cent. An analysis of the phenomena in the serums of 372 cases suffering from a variety of pathological conditions will be considered in a later paragraph.

There are a number of factors which influence the rate of filtration. There is an increasing rate of filtration with increasing age. This is most manifest in the band 43 to 50. In this band under twenty years of age the averaged rate is 3.6 per cent, from 20 to 40 years it is 4.1 per cent, from 40 to 60 years it is 4.6 per cent and over 60 years it is 4.7 per cent per 100 Å units. In general the serums from males filter out slightly more light than those from females, thus in type A the rate for females is 3.9 per cent; for males 4.6; in type B the rate for females is 2.3 per cent; males, 2.5; while in type C the rate for females is 4.8 per cent; males, 5.8.

Increasing the elapsed time between taking the specimen and examining it, may alter the type of reaction, and increases the rate of filtration. As illustrative, a given serum when fresh had an A curve with an averaged filtration rate per 100 Å units of 0.5 per cent; twenty-four hours later the reaction type was A and the averaged rate of filtration 1.4 per cent; forty-eight hours after taking the specimen the reaction was of the B type and the filtration rate was 1.5 per cent.

Radiation of the serum with ultraviolet light, one skin erythema dose being given and the serum being cooled with a fan, increased the filtering power of the serum and occasionally changed the type of reaction.

Inactivation of complement, as is done in the test for complement fixation, often changed the type of reaction and either increases or decreases the filtering power. Further investigation showed that those serums containing natural amboceptor had an increased filtering power after inactivation, while in those serums without natural amboceptor the filtering power decreased after inactivation. No mathematical correlation could be established between the amount of natural amboceptor present and the degree of increase in the filtration rate. As typical of

the change two instances are cited. In one serum without natural amboceptor the initial filtering rate was 2.7 per cent, which after inactivation fell to 2.3 per cent. In another specimen containing natural amboceptor the initial rate was 2.6 per cent which after inactivation rose to 3.1 per cent.

The constancy of the filtration rate in the same individual was studied in ten cases, specimens being taken at from four to thirty-five day intervals. It was found that in those individuals in whom the clinical condition remained unchanged the filtration rate was constant, thus in a case of chronic productive arthritis the initial rate was 3.7 per cent seven days later it was 3.8 per cent; a case of neurasthenia had an initial rate of 2.8 per cent and twenty-two days later 2.7; a case of senile dementia had an initial rate of 2.1 per cent and four days later the same rate. In none of these patients had there been any decisive change in the clinical condition during the period of observation. In contrast, patients in whom there had been a decided change in the clinical condition showed marked changes in the filtration rate. In a case of acute appendicitis the inital rate was 1.7 per cent, fourteen days later when clinically improved, 2.2 per cent. In a case of fibroid of the uterus, the inital rate was 3.7 per cent, twelve days later (three days post-operative), 1.9 per cent. In a case of inguinal hernia, the initial rate was 3.1 per cent, eleven days later (five days post-operative) 1.3 per cent. While this phenomena will be the subject of a later communication describing its application in prognosis it may be summarized here that as the clinical condition improves the rate rises, and as the clinical condition gets worse the rate falls.

With five serums the total protein content of each was determined directly after the readings, while with five others the readings were followed by determination of the lipoid content. No relationship between the amount of light filtered out or the reaction type and either the protein or lipoid content could be demonstrated.

In other observations it was noted that if definite amounts of either normal acid, or alkali, were added to definite amounts of serum that there were changes both in the type of reaction and in

the degree of filtration. In general the addition of either acid or alkali decreased the rate of filtration, the decrease in the filtration rate averaging four-tenths of one per cent.

A statistical summary of the light filtering power of serum in 372 cases of various disease conditions is presented in table 1. The wide range of the reaction when individual serums were compared rendered it impossible to demonstrate consistent variations between individual serums, but when groups were averaged certain trends of reaction became apparent. It is believed that group averaging is permissible since light filtration is a physico-biological process in which for the present we are interested in determining trends rather than estimating the mathematical degree of change.

The various conditions studied have been rather broadly grouped and individual diagnoses are not given. It was noted that the percentage of each group in the various types of reaction varied. Thus 40 per cent of the normal individuals gave a type A curve while in the pathological serums the lowest rate of type A curve was in the benign tumors which gave 13 per cent, and the highest rate was in pregnancy which gave 58 per cent. Normal serums showed 20 per cent to give type B curve while with pathological serums the lowest rate was given by acute bacterial infections (10 per cent), the highest rate (39 per cent) being given by benign tumors. With the type C curve the normals gave 40 per cent while the lowest rate of the pathological sera (30 per cent) was found in a group of endocrine dysfunctions, the highest rate (61 per cent) occurred in a group of mental cases.

If the filtration rate in each reaction type and for each diseased group be compared with the normal, variations are again observed but such variations occur without any definite rule. In the type A reaction all of the group averages with the exception of endocrine dysfunctions, and acute and chronic bacterial infections, showed a higher filtration rate than the normals. With the type B reaction, all the groups except the mental cases showed lower filtration rates than the normals. With type C curve a still different arrangement of the groups occurred.

It is evident therefore that no diagnostic significance can be attached to a determination of the light filtering power of serum, although as has been previous suggested there is apparently a definite aid in prognosis associated with its determination.

SUMMARY

It is apparent that human blood serum has the power to filter out light in the range 7200 to 4300 Å. This filtering action varies with different serum both in intensity and point of maximum filtering power. The various factors which affect the filtering power, namely, age, sex, heat, age of specimen, ultraviolet radiation, and disease states, plus the observation that protein and lipoidal content of the sera are not in relation to the filtering power, suggests that the filtering power is dependent upon the colloidal state of the serum. The nature of the changes in the colloidal state can at the present be only a matter of theory since experimental observations are lacking.

I beg to express my appreciation to Miss Miriam Berke for technical assistance.

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PERMANENT COLOR STANDARDS FOR FOLIN'S FERRICYANIDE SUGAR METHOD

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Folin's[1,2] ferricyanide method for blood sugar estimation has been found admirably applicable to the determination of sugar in spinal fluids. This method has the distinct advantage of requiring only 0.1 cc. of spinal fluid, whereas the Folin-Wu copper method requires 2 cc. In order to make this ferricyanide method rapidly and intermittently available for general clinical and hospital practice in the estimation of spinal fluid sugar, it seemed desirable to devise a permanent standard whereby the unstable sugar standard and the colorimeter could be eliminated. Standards so prepared furnish a rapid means for sugar measurement in spinal fluids comparable to the accepted colorimetric procedures for icterus index and the phenolsulphonephthalein tests.

Folin's method is essentially the reduction of an alkaline solution of potassium ferricyanide by the sugar and the precipitation of the resulting potassium ferrocyanide as Prussian blue by the addition of ferric iron. The precipitated Prussian blue is kept in a colloidal suspension by the use of gum ghatti. Sugar values vary with the depth of the blue color produced.

METHOD OF PROCEDURE

The technique as used by Folin and Malmros[3] was followed with the exception that 1 cc. of a 0.2 per cent solution of potassium ferricyanide, as originally recommended, was used rather than 2 cc. of a 0.4 per cent solution because a better range of color is obtained in the lower end of the scale with the use of the more dilute ferricyanide solution. This is especially desirable when the method is applied to spinal fluids since the amount of sugar found in normal spinal fluid is relatively low, from 50 to 70 mgm. per cent.

Reagents

1. Dilute Tungstic Acid Solution.

Transfer 20 cc. of a 10 per cent sodium tungstate solution to a liter volumetric flask. Dilute to a volume of about 800 cc., add 20 cc. of 2/3 N sulphuric acid and then dilute the whole to one liter.

2. Potassium Ferricyanide Solution, 0.2 per cent.

Dissolve 1 gram of potassium ferricyanide C.P. in distilled water and dilute to 500 cc.

3. Sodium Cyanide-Carbonate Solution.

Dissolve 8 grams of anhydrous sodium carbonate in 50 cc. of water. Add 150 cc. of a 1 per cent solution of freshly prepared sodium cyanide solution. Mix and dilute to 500 cc.

4. Ferric Iron Solution.

Fill a liter cylinder with distilled water. Suspend on a copper wire screen, just below the surface of the water, 20 grams of soluble gum ghatti* and allow to stand over night. Remove the screen and strain the liquid through a double layer of cheese cloth. Dissolve 5 grams of anhydrous ferric sulfate in a mixture consisting of 75 cc. of 85 per cent phosphoric acid and 100 cc. of distilled water and add this solution to the solution of gum ghatti. Add sufficient 1 per cent potassium permanganate solution to destroy the reducing materials present in gum ghatti so that a slight pink color will persist for about five minutes (between 15 and 40 cc. will be required). Care should be taken that there is no pink color present in the reagent when used.

5. Stock Glucose Solution.

Dissolve 1 gram of benzoic acid in 200 to 300 cc. of water. Add exactly 1 gram of glucose C.P. and dilute to 500 cc. One cubic centimeter of this solution contains 2 mgm. of glucose.

6. Standard Glucose Solution.

Dissolve 0.25 gram of benzoic acid in 500 to 600 cc. of water, add 5 cc. of the stock solution of glucose (reagent 5) and dilute to 1 liter. One cubic centimeter of this solution contains 0.01 mgm. of glucose. (These sugar solutions will not be needed unless it is desired to check the permanent color standards.)

Technique of the Determination

Pipette 10 cc. of the dilute tungstic acid solution into a centrifuge tube. Add exactly, from a 0.1 cc. capillary pipette, 0.1 cc. of spinal fluid (or blood) to the tungstic acid solution. Care should be taken to first wipe off the outside of the pipette with a piece of filter paper so that exactly 0.1 cc. is measured. Mix well and then centrifuge for about five minutes. Measure exactly 4 cc. of the supernatant fluid into a Pyrex tube. Add 1 cc. of the 0.2 per cent solution of potas-

* Soluble gum ghatti may be obtained from Arthur H. Thomas Co., Philadelphia or Eimer and Amend, New York City.

sium ferricyanide and 1 cc. of the cyanide-carbonate solution. Heat in a boiling water bath for exactly eight minutes. Cool immediately in a cold water bath for about two minutes, add 5 cc. of the ferric iron solution, mix and let stand for five minutes. Add sufficient water to bring the total volume of the mixture to 25 cc. and compare with the permanent color standards directly.

In the case of pathological conditions such as diabetes mellitus where the sugar content is high it is only necessary to dilute the color formed with a diluting solution prepared from the stock reagents. If the color is above the highest standard, remove an aliquot part of the prepared colored sample and dilute to 25 cc. with a freshly prepared diluting solution made by adding 4.0 cc. of 0.2

TABLE 1

PREPARATION OF PERMANENT COLOR STANDARDS*

SUGAR**	20 PER CENT COPPER SULPHATE $CuSO_4 \cdot 5H_2O$	5 PER CENT CHROME ALUM $Cr_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$	0.5 PER CENT GOLD CHLORIDE $AuCl_3 \cdot 2H_2O$	2.4 PER CENT COBALTOUS CHLORIDE $CoCl_2 \cdot 6H_2O$
mgm. per cent	*cc.*	*cc.*	*cc.*	*cc.*
0	0	0.2	2.3	0
10	0.1	0.2	2.0	0
20	0.5	0.4	1.8	0
30	0.9	0.6	1.3	0
40	1.5	1.0	1.0	0
50	2.1	1.7	0.6	0
60	2.8	2.0	0.3	0
70	4.2	2.3	0	0.7
80	6.8	2.1	0	2.5
90	9.5	2.0	0	3.5
100	12.0	2.0	0	4.0

* Each tube contains 2 drops of concentrated HCl and the total volume is made to 25 cc.

** Standards read in milligrams per cent if 0.1 cc. of spinal fluid is used and if 4 cc. of the clear supernatant centrifugate is taken.

per cent ferricyanide solution to 20 cc. of gum ghatti iron solution and diluting to 100 cc. By utilizing a suitable aliquot of the final solution it is possible to measure as much as 200 mgm. per cent of sugar. If more than this amount is present it becomes necessary to use a smaller aliquot of the tungstic acid centrifugate diluted to 4 cc. in order to keep the sugar concentration within the oxidizing limits of the potassium ferricyanide present. By the use of these dilution methods it is possible to measure sugar values from 10 to 500 mgm. per cent with the use of the permanent color standards.

Experimental preparation of standards

Known quantities of a freshly prepared standard sugar solution (1 cc. containing 0.01 mgm. of sugar) were used to set up a series of tubes representing

from 0 to 100 mgm. per cent of sugar when 0.1 cc. sample of spinal fluid is taken. Efforts were then made to match these colors with synthetic solutions consisting of varying amounts of copper sulfate, potassium dichromate, chrome alum, and cobaltous chloride. It was found possible by a cut and try method to match these colors with mixtures of these solutions. However, after some length of time, it was observed that in the lower part of the range the yellow tint had diminished. Substitution of a gold chloride solution for potassium dichromate was found to be satisfactory. Two drops of concentrated hydrochloric acid were added to each tube to prevent the separation of metallic gold and the tubes then sealed to prevent evaporation. These standards have kept for four months. Table 1 gives the amounts of each of the four solutions needed to make up the standards.

Pyrex test tubes may be used for running the determination. For comparison the solutions should be transferred to clear glass tubes of the same diameter as those in which the standars are kept. In this laboratory 25 cc. clear glass cylindrical ampules 2.25 cm. in diameter were used. The ampules were viewed from the side against a white background. It is possible to interpolate values between the standards. Differences of 5 mgm. per cent can be very easily seen.

PERMANENT COLOR STANDARD AND COLORIMETER READINGS COMPARED

Comparisons of the amount of sugar found in spinal fluids were made on a number of samples first by matching with the permanent color standards and then by comparison with a known sugar standard representing 50 mgm. per cent in a colorimeter. A 50 instead of a 100 standard was used since it is nearer the range of the sugar content of normal spinal fluids.

The sugar values on thirty-eight spinal fluids were determined by these two methods. Considering the colorimeter readings as valid, the permanent standard results on twenty of these fluids showed a variation of $\pm$ 1.0 mgm. per cent. Eleven samples gave results varying between $\pm$ 1.0 and $\pm$ 2.0 mgm. per cent and six of them gave slightly greater variations. In only one case the value obtained with the use of the permanent standards was 4.0 mgm. per cent higher than that obtained by the colorimeter.

In another set of experiments sugar determinations were made by the Folin-Wu copper molybdate method[4] at the same time as the Folin ferricyanide method on fifteen different samples. A variation between the two methods was found amounting for the most part to a difference ranging from 0.8 to 3.4 mgm. per cent.

In three samples differences of 4.4, 5.3, and 5.4 mgm. per cent were found. In ten out of the fifteen samples the values found with the permanent standards were between the results obtained by the two colorimeter methods. Results show that the amount of sugar obtained with the Folin-Wu copper method and the amount obtained with the Folin ferricyanide method, either with the use of the permanent color standards or the colorimeter, does not vary greatly.

It is believed that the use of permanent color standards is feasible in the determination of sugar by the Folin ferricyanide method. It eliminates the undesirable features incident to the use of a sugar standard and also the expense involved in procuring a colorimeter. While the results obtained by simple visual comparison of color are not as accurate as those obtained by mechanical measurement, the results in either case are more accurate than necessary from the standpoint of clinical interpretation. The ease and rapidity with which a determination is made by the use of permanent color standards should commend their use to clinical practice.

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A COMPARISON OF THE RELATIVE VALUES OF THE INTRACUTANEOUS SKIN TEST AND OF THE PATHOGEN-SELECTIVE CULTURE IN SELECTING BACTERIA FOR VACCINES FROM MIXED CULTURES*

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In discussing vaccines, Kolmer and Boerner[2] enumerated eleven general principles of which the last two are as follows:

10. The pathogen-selective[1, 3] method (given below) may be helpful in selecting bacteria for vaccines in mixed infections on the basis that only those capable of growing in the whole coagulated blood of the patient are apt to be virulent. It is possible, however, that an organism unable to grow in the blood or produce septicemia may still be able to produce local infection.

11. Intracutaneous skin tests are also advocated for the selection of bacteria in the preparation of vaccines on the principle that only those yielding positive reactions should be employed. Positive skin reactions may be an indication of acquired allergic sensitization and therefore evidence of infection with the organism; they may be also plain inflammatory reactions if the inoculum contains a toxin or toxins for which there are no or insufficient amounts of antitoxin in the blood. The exact value or status of the method is as yet unknown.

In mixed cultures, in order to test the value of intracutaneous tests as a means of differentiating those organisms that are capable of infecting the patient from those against which he possesses good resistance, the reactions produced by the intracutaneous injections of those organisms that grew in the patient's fresh, whole, coagulable blood were compared with the reactions produced by those organisms that were killed by this blood.

METHOD

The patients cultured and tested were in the pediatric ward of the Mt. Sinai Hospital, Philadelphia. The bacteriological work was done in the hospital laboratory by Miss Marian Barnes.

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* Read at a joint meeting of the American Society of Bacteriologists and Pathologists and the American Association of Immunologists, April 28, 1932.

Twenty children were thus studied, and also eight adults for whom pathogen-selective vaccines containing but a single organism were made by Dr. Fred Boerner, in the Laboratory of the Graduate Hospital of the University of Pennsylvania.

A swab containing the inoculum first was rubbed on the bottom of a sterile test-tube, into which one or two drops of broth had been placed, after which the swab was inserted in a tube of hormone broth medium. About 5 cc. of the patient's fresh, whole, coagulable blood were then placed in the first inoculated tube. After twenty-four hours incubation the tubes were subcultured on blood-agar. Each organism present in the subcultures was then grown in pure culture in a hormone broth medium. After four days growth in the incubator enough 5 per cent solution of tricresol was added to the pure culture of each organism to make 0.5 per cent of the total volume. The following day each vaccine was cultured for sterility and if found sterile was placed in a small ampule. The ampules therefore contained all the organisms that grew in the patient's blood owing to the lack of bactericidal power against them in the blood, and also all those organisms that failed to grow in the patient's blood, because of the presence in it of bactericidal power against them. The exogenous toxins produced by the contained organism also were in each ampule. The vaccine in each ampule was injected intracutaneously in the forearm of the patient from whom the organisms were obtained.

Eighty-four organisms were studied, sixty-three that grew in the patient's blood and twenty-one that failed to grow in the patient's blood.

In studying the reactions the areola and induration were charted separately, inasmuch as there seemed to be little correspondence between them, as will be seen from the following summary:

The areola and induration was of the same degree in 32 per cent of tests with organisms that grew in the patient's blood and 9 per cent with those that failed to grow in the blood; the areola was more marked than the induration in 49 per cent of the tests with organisms growing in the patient's blood and 86 per cent of those not growing; the induration was more marked than the areola in 19 per cent of the tests with the organisms growing in the patient's blood and 5 per cent of those not so growing. Thus the induration was of the same degree or of greater intensity in 41 per cent of tests with organisms growing in the patient's blood and 14 per cent of those not growing in the same blood.

A comparison of the areolas produced by intracutaneous injection of organisms that grew and of those that failed to grow in the patient's blood is given in table 1. No difference between the two groups was noted in areolas of slight, moderate or marked intensity. A larger percentage of the still stronger reactions, however, was produced by the organisms that failed to grow in the blood, but in no instance did the latter produce a very mild or no areola, although these results occurred in 13 per cent of the injections of organisms that grew in the patient's blood.

TABLE 1

COMPARISON OF DEGREES OF AREOLAS AND INDURATED AREAS IN INTRACUTANEOUS TESTS WITH VARIOUS ORGANISMS

DEGREE OF REACTION	AREOLAS				INDURATED AREAS			
	Bacteria growing in patient's blood		Bacteria not growing in patient's blood		Bacteria growing in patient's blood		Bacteria not growing in patient's blood	
	number	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*
−	6	10			10	16	1	5
±	2	3			1	2		
+	10	16	2	9.5	16	25	7	33
++	14	22	4	19.0	26	41	11	52
+++	15	24	7	33.0	7	11	2	10
++++	11	17	5	24.0	3	5		
+++++	5	8	2	9.5				
++++++			1	5.0				
− and ±	8	13			11	18	1	5
+ to +++	39	62	13	61.5	49	77	20	95
++++ to ++++++	16	25	8	38.5	3	5		

The intensity of the induration produced by the two groups is contrasted in table 1. Little difference is shown except that a greater per cent showing no induration, followed the injection of organisms that grew in the patient's blood.

In table 2 the different organisms are indicated with the various types of reaction they produced, those that grew in the patient's blood being recorded separately from those that were killed by the blood.

The amount of time required for the reaction to reach its maximum intensity was studied to see if there was any difference

between the two groups, but none was found. Approximately the same per cent of reactions reached their maximum intensity both for the areola and the induration, whether the organisms tested grew in the patient's blood or not, either within a day or within two or more days. About twice the per cent of areolas

TABLE 2

ORGANISMS	AREOLAS										INDURATED AREAS									
	Bacteria growing in patient's blood					Bacteria not growing in patient's blood					Bacteria growing in patient's blood					Bacteria not growing in patient's blood				
	− or ±	1+	2+	3+	4+ to 6+	− or ±	1+	2+	3+	4+ to 6+	− or ±	1+	2+	3+	4+ to 6+	− or ±	1+	2+	3+	4+ to 6+
Streptococcus (green producing)			2	1			2	1	3			2	1				3	3		
Streptococcus (hemolytic)	1	2						1	2			1	2					1	2	
Streptococcus (sl. hemolytic)								1										1		
Streptococcus anhemolyticus	2		2	4			1	2		2	1	2	5			1	1	3		
Streptococcus (anaerobic)							1											1		
Staphylococcus aureus	1	1	1	1	2		2	1	4	6	1	1	3	1		1	2	8	1	1
Staphylococcus albus	3		2		5				3	2	1	3	4	2		2	2			1
Pneumococcus			1	1	1			2	1	1	1	2					1		2	1
Gaffkya (Micrococcus) *tetragena*									1									1		
Neisseria sicca							1			1							1		1	
Neisseria flava		1										1								
Escherichia coli (B. coli)					1			1		1	1						1		1	
Pseudomonas aeruginosa (B. pyocyaneus)								1										1		
Klebsiella pneumoniae (Friedlander's bacillus)						1											1			
Hemophilus influenzae					1									1						
Diphtheroids								1										1		
Totals	7	4	8	7	10	1	7	11	14	13	5	12	15	4		7	11	20	5	3

reached their maximum degree of reaction at the end of one day as those that became maximal at two days or more. Of those showing induration, about 50 per cent were maximal within twenty-four hours.

The duration of the reaction was also studied. The results showed there was practically no difference in the duration of the

reactions produced by those organisms growing and those failing to grow in the patient's blood. In both groups approximately half of the areolas persisted for three days or less, the rest lasting five days or more. Slightly more than half of the indurated areas resolved within three days, while the remaining ones lasted five days or more.

CONCLUSIONS

(1) There is practically no difference between the reactions produced by intracutaneous injections of organisms that are able to grow in the patient's fresh, whole, coagulable blood and the reactions produced by organisms that are killed by his blood.

(2) There seems to be no correspondence between absence of bactericidal power in the blood of the host against a given organism and production in that host of a positive reaction by the intracutaneous injection of such organism.

(3) There probably is no relationship between hypersensitiveness in the host to the exogenous and endogenous toxins of a given organism and the pathogenicity of such organism for that host.

(4) It is questionable whether intracutaneous tests can identify, in a mixed culture, the bacteria that are infecting the patient.

(5) Therefore, intracutaneous skin tests are probably unreliable for selecting bacteria in the preparation of vaccines.

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THE HISTOPATHOLOGY OF METHYL ALCOHOL POISONING*

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One of the earliest references to the toxicity of wood alcohol is in an article published by MacFarlan[14] between the years 1855 and 1856 in London. Approximately twenty-five years later a French investigator, Poincaré,[18] carried on experimental work upon the toxic effect of its vapors. From that time to the present literature contains many articles concerning the toxicity of this substance. As early as 1880 it was found to be toxic regardless of its portal of entry and also that the toxic manifestations were widespread throughout the body. Even Poincaré mentioned such changes in the liver, heart, kidneys, and lungs of his experimental animals. Holden[9] observed degenerations of the ganglion cell layer of the retina (with Nissl stain), and degenerations of the medullary sheath affecting some fibers of the optic nerve. These changes were accompanied by numerous hemorrhages into the meninges of the cord and brain.

Following the development of the ophthalmoscope, clinical observation of the eye grounds became more common, and the changes in this organ grew in importance until methyl alcohol became generally considered as a specific toxin for the retina and in particular for the optic nerve. This is exemplified in the statement of Wood and LaWall,[27] "that this predilection for the optic nerve is but a part of the general tendency toward nerve trunks," and that of Tyson,[23] who stated that while these toxic manifestations are most pronounced in the retina and optic nerve, possibly other cranial nerves are also affected.

The tissues forming the basis of the present study resulted from

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6–9, 1932.

a series of experiments made by Dr. Carey P. McCord[15] in the Industrial Health Conservancy Laboratories of Cincinnati, Ohio, in which a careful study of the toxic effects of methyl alcohol following various methods of administration was made. A very brief summary of that work is as follows.

The animals used were thirty-one young rhesus monkeys, fifty-eight rabbits of four breeds, and 176 white rats. Of these the final number having tissues available for microscopic study were forty-one monkeys, twenty-six rabbits, and eighty-one rats. These animals were treated by skin absorption, inhalation, and ingestion. The materials used were various grades of natural and synthetic methyl alcohol, samples of which had been obtained both from the manufacturer and upon the open market. Details concerning the manner of application can be found in Dr. McCord's discussion.

PRESENT STUDIES

No animal was utilized for tissue study unless found before the body warmth had left the corpse. Autospies were performed immediately and portions of tissue preserved in the proper fixatives. The remainder of the organs was tested chemically for the presence of methyl alcohol or its decomposition products.

The eyes of all monkeys and all rabbits except albinos were examined at least once by a competent ophthalmologist.

Intoxication was evident following all methods of application. The first sign of intoxication was a dilatation of the pupils, which reacted slowly to light. This was followed by an illness which sometimes led to prostration and possibly to death. Recovery from the effects of methyl alcohol frequently took place even during sustained exposure. Animals which had been blind upon ophthalmoscopic examination frequently regained sight and a subsequent examination revealed no pathology in the eye grounds. The brand or purity of the alcohol seemed in no way to affect the pathological changes. Animals subjected to methyl alcohol have regularly shown the presence of this substance in the blood, urine, and tissues. Formaldehyde was never recovered in any appreciable amounts.

By inhalation the threshold of danger is well below 1000 p.p.m. of methyl alcohol vapor, while by skin absorption the threshold is near 0.5 cc. per kilogram of body weight applied four times daily. Individual animals vary greatly in susceptibility, black animals being extremely resistant. There is some species variation in that rats are most susceptible and rabbits most resistant.

The typical histopathological picture of the tissues of these animals following methyl alcohol poisoning varied more with the species of animal used than upon the method of administration. Upon repeated contacts of the skin to alcohol it showed evidence of irritation, which sometimes changed to actual ulceration and necrosis. Irritation of the skin was noted by Rosenau[20] in individuals poisoned by methyl alcohol. Dublin and Lieboff[4] mentioned inflammation of the throat and mucous membranes of the air passages among the clinical manifestations of the poisoning following inhalation of methyl alcohol fumes.

Following either method of administration the organs of the abdomen most constantly affected were liver, spleen, and kidneys. The reaction in the liver was practically always one of parenchymatous degeneration, which, in the more severe cases, had proceeded to focal necrosis. This change in the liver cord cells was much more constant than fatty degeneration spoken of by Poincaré in 1879, and since then by Dublin and Lieboff, Smith,[22] Weese,[26] and others. The almost constant change in the kidney was a parenchymatous degeneration of the epithelium lining the convoluted tubules. This finding was also confirmed by Poincaré, Weese, Smith, and by Isaacs.[10]

In the thoracic cavity both the lungs and heart showed rather constant evidence of pathological change. In some cases the lungs gave evidence of a terminal pneumonic consolidation as noted by Weese in mice. In the milder cases edema, congestion, and desquamation of alveolar epithelium was noted, as mentioned by Pierce and by Poincaré. These changes were observed more frequently in those animals subjected to inhalation of alcohol fumes than in those treated by either absorption or by oral administration. The earliest changes in the heart began as an edema and progress to granular degeneration, and in some in-

stances, to a final necrosis of the heart muscle fibers. These findings also confirmed those of Poincaré.

The hemopoietic system and blood showed interesting changes. The earliest reference found to any change in the blood and blood vascular system is in a paper by Buchner, Fuchs, and Megele,[2] published in 1901, in which they discussed the capillary engorgement produced by the external application of methyl, ethyl, and propyl alcohols. Twelve years later Miura[16] injected methyl alcohol into the blood stream of four rabbits and one dog. From his results he concluded that methyl alcohol is toxic to the blood-forming organs. The next year, after experimental poisoning by inhalation, Tyson and Schoenberg[25] stated they believed methyl alcohol to be a true hematoxic. All the degenerative changes observed in other tissues they explained on the basis that the interference with the circulation of the blood deprived them of of their nutrition and oxygen. In Smith's recent paper he mentioned splenic engorgement and phagocytosis.

In the present series the lymph nodes were frequently found to be hyperplastic and the spleen in many cases gave evidence of increased blood-forming activity. This phase of the toxic action of methyl alcohol opens a somewhat new and interesting field of investigation upon this subject and upon which we hope to make further report at an early date.

The influence of methyl alcohol upon the central nervous system was much the same as elsewhere in the body: capillary congestion, edema, and patchy degeneration in the neurones. This cellular degeneration occurred more often in the spinal cord than in the brain. Pierce recorded cerebral congestion occurring both in his own cases and in those of Strohmberg. Isaacs noted in his collection of human cases edema and congestion of the brain and meninges and an increase in the amount of spinal fluid. Rühle[21] found in dogs, scattered hemorrhages along the blood vessels in the pons, medulla, and cord. He also noted that the vascular endothelium contained large amounts of lipoid and that there was perivascular infiltration. This condition preceded the hemorrhages. In the experiment under discussion there was no evidence of either the perivascular infiltration or hemorrhages noted

by this investigator. Holden also described hemorrhages into the meninges, brain, and cords of dogs. He agreed with several other investigators that the alcohol causes pathological changes in nerves other than the optic nerve. He reported that the experimental dogs appeared partially deaf as well as blind before death. Both Rosenau and Tyson refer to hearing impairment in human cases. Tyson also cited one case of paresis of the left internal rectus muscle of the eye. Jelliffe[11] has noted other forms of neuritis than optic neuritis, citing a human case with neuritis of the upper extremities. McCord, in his clinical observations on the animals which comprise this study, noted evidence of such peripheral nerve involvement. These clinical observations were later confirmed in stained preparations of peripheral nerve of these animals.

In studying the eye and optic nerve constant changes were found both in retina and nerve. However, the changes in the retina predominated and were uniformly of the nature of an acute toxic lesion. The vessels of the choroid were markedly congested. The entire retina was edematous, but especially the fiber and ganglionic cell layers. The ganglion cells were degenerated. This degeneration was patchy in occurrence, normal areas being immediately adjacent to markedly degenerated ones. These degenerated areas were not confined to any one area of the retina but were scattered throughout. These retinal changes are quite similar to those found by MacDonald[13] in human cases, and by Smith, Weese, Holden, Friedenwald,[5] Tyson and Schoenberg,[24] and Birch-Hirschfeld[1] in experimental animals. Hale[6] discussed Moulton's two cases of complete optic atrophy without changes in the retina. On the other hand it seems not uncommon to find degenerations in the retina without any degenerative changes in the optic nerve. Birch-Hirschfeld definitely stated that degenerations in the optic nerve occurred in but one of his cases. MacDonald did not find any optic nerve pathology in his human cases. Weese and Tyson and Schoenberg fail to mention the optic nerve. Very few of our animals showed any change in the optic nerve although numerous specific neurological stains were used. The animal showing such changes most definitely was the monkey which was blind at death.

With these findings in mind the discussion arises as to whether the retina or optic nerve is affected first, or whether they might both be the result of the same change and occur simultaneously. Tyson believed that the loss of function is due to the affected nerve fibers first or at least coincidentally with the degeneration in the ganglion cell layer. However, our observations in the present study force us to agree with Birch-Hirschfeld, MacDonald, and Henderson and Haggard[7] in thinking that the degeneration of the retinal ganglion cell uniformly precedes any degenerative changes in the optic nerve.

The most important factor in this process is the direct action of the alcohol itself, while a contributing factor may be the accompanying edema. That such a rôle might be played by edema is suggseted in a bulletin published by the National Research Council of Canada.[19] Kasaas[12] believed that circulatory disturbances in the choroid and in the sheath of the optic nerve, together with edema, lead to degeneration of the retina. The author stated that the edema disappeared and visual power partially returned if collateral circulation be restored. In human cases of a chronic nature, several of recurrent transitory blindness with a gradual impairment of sight are reported in a bulletin issued by the New York State Department of Labor,[3] and in some animals of the present series blindness was apparently transitory. One monkey in particular was blind both clinically and upon ophthalmoscopic examination at one time during the experiment, but prior to death its sight was proved again normal by the same means. Histological examination of the eyes of this animal revealed no evidence of pathologic change. Such transitory blindness was not of uncommon occurrence among the animals reported by McCord.

There has been a gradually growing tendency to consider methyl alcohol as a specific poison to the optic nerve and retina. This concentration of attention upon one manifestation of a disease to the exclusion of its other less conspicuous lesions is not confined to this disease, however. Among rather numerous possible examples we may cite the fact that until the very recent careful observations of Herrick[8] the thought prevailed that the

pathology of meningococcic infection was limited entirely to the meninges. The proof that such infections are true cases of generalized septicemia has thrown new light upon this disease and explains the not infrequent overwhelming infections of this organism and sudden deaths occurring before meningeal pathology has developed. Other conditions in which a similar attitude has been assumed by the physician are the relationship of the kidney to hypertension, and also in the lesions of poliomyelitis. Similarly the lesions of the eye have been so conspicuous as to mask the other manifestations of the poison of methyl alcohol. The pathological conditions arising from these associated effects may be, however, just as constant and possibly more important than the changes taking place in the eye. Also it is possible that an individual may suffer from methyl alcohol poisoning in severe degree and still show no eye changes, just as meningeal lesions are no longer considered essential for the diagnosis of meningococcus infections.

SUMMARY

A histopathological study has been made of forty-one monkeys, thirty-six rabbits, and eighty-one rats which had previously been treated with methyl alcohol. The alcohol was administered by inhalation, by skin absorption, and orally. The method of application made no apparent difference either in the type of lesions or in their severity.

There is some variation in species susceptibility to methyl alcohol in the animals studied; rats are most susceptible to the alcohol and rabbits are most resistant. Individual variations in susceptibility to this toxin are very marked.

The characteristic pathological lesions of methyl alcohol poisoning are degenerative ones. Only the parenchymal tissues and neurones are affected. This granular degeneration of parenchymal tissue may proceed to necrosis. There is practically never injury to connective tissue and therefore seldom any fibrosis. These changes are accompanied by a generalized capillary engorgement and edema.

The pathological conditions in the eye are identical with those

found elsewhere in the body. The edema is most marked in the retina and optic disc. Degeneration of the ganglion cells of the retina is sometimes followed by degenerative changes and fibrosis within the optic nerve.

There may be multiple degenerations involving nerves other than the optic nerve.

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EDITORIAL

The Bendien Test

Bendien's book, *Spezifische Veränderungen des Blutserums*, published in 1931, was translated the same year by A. Piney. There may be academic interest in Bendien's method of approach to various problems. However, his chief claim for recognition is on the grounds that his test has value in the diagnosis of cancer. Bendien's reagents are made up with varying quantities of tenth normal acetic acid, and of tenth normal sodium vanadate. These various mixtures provide twenty different solutions with very slight differences in hydrogen ion concentrations from pH 3.3 to 5.4. Five cubic centimeters of each reagent is used in the test, which requires twenty tubes. In each of these tubes, before adding the reagent, there is placed 0.5 cc. of clear serum and 0.5 cc. of distilled water. After standing twenty-four hours at room temperature flocculation is found to occur in some of the twenty tubes. In certain diseases, notably carcinoma, active tuberculosis, or obstructive jaundice, precipitation may occur in the first or second tube, pH 3.3. As the solutions become slightly less acid, precipitations occur with serums from patients with other diseases, diabetes, pleurisy, pregnancy, malaria, nephritis, furunculosis, scarlet fever, syphilis, and so forth. Bendien was not satisfied with such a simple procedure as that briefly outlined but increased the technical difficulties by filtering out some of the precipitates, weighing the residue and making a 1 per cent $NaHCO_3$. This was then studied spectrophotometrically.

Meyer reported unfavorably on the test and stated that it is not specific in any way. Smith, Holiday and Marrack investigated the spectrophotometric details of the test and concluded that the method is valueless in the diagnosis of cancer. Such was also the conclusion of Freeman. A recent advocate for the test, Lowe, has complicated the technic further by studying reactions of heated and unheated serum and by dissolving the precipitate in hundredth normal sodium hydroxide and then using a Löwe interferometer. It is not necessary to discuss the some-

what involved estimations that must then be made by means of which a "reaction formula" is evolved, placing the serum either in a malignant or nonmalignant category. Lowe referred to criticisms of Bendien's original method and claimed superiority for his own method. It does not seem, however, that Lowe has entirely answered the criticism of Fine, who stated: "The Bendien chemical test has no real value in diagnosis. It has certain value in prognosis, probably not greater than that of the clinical signs and symptoms present. In its mechanism and in the information it supplies it is identical with the sedimentation rate test, to which it has not been proved superior." Fine's explanation for the early reports of 100 per cent accuracy in the diagnosis of cancer is: "when a batch of bloods is supplied containing a certain number derived from inoperable carcinomas, the remainder being from mild, non-wasting, and non-suppurative conditions, those from carcinoma alone give a precipitate in tubes 1 and 2. Such success, though of no practical application, produces an ephemeral enthusiasm for the test—a phase I passed through myself."

It is of course greatly to be desired that some sharply differential test be devised for the early diagnosis of cancer. It is barely possible that Lowe's modification of Bendien's test may have made this method more useful, but the weight of the evidence is still on the side of those who hold the opinion that this method cannot yield specific results.

A. H. SANFORD.

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NEWS AND NOTICES

The Twelfth Annual Convention of the American Society of Clinical Pathologists

The Twelfth Annual Convention of the American Society of Clinical Pathologists was held in Milwaukee, Wisconsin from June 9 to 12. The meetings were well attended and as was anticipated the Symposium on the Medico-Legal Autopsy was a tremendous success. Due to popular demand it has been decided to issue the Symposium as a monograph after the papers have been published in the JOURNAL. The rest of the Scientific sessions contained papers of great merit and many valuable contributions were made.

The details of the business meeting will be published in a subsequent issue of the JOURNAL.

The following officers were elected and committees appointed:

President-Elect: Dr. F. H. Lamb
Vice-President: Dr. J. J. Seelman
Secretary-Treasurer: Dr. A. S. Giordano
Members of the Executive Committee:
Dr. W. M. Simpson (3 years)
Dr. H. C. Sweany (3 years)
Dr. J. H. Black (1 year)
Members of the Board of Censors:
Dr. J. J. Moore (3 years)
Dr. A. V. St. George (2 years)
Dr. C. W. Maynard (3 years)
Members of the Board of Registry:
Dr. R. R. Kracke (3 years)
Dr. H. H. Foskett (3 years)

The following were elected to membership:

Ball, Howard A.
Balle, Alfred L.
Barret, Harvey P.
Bates, Lewis Beals
Bleyer, Leo Frederick
Braunstein, Wm. P.
Bugher, John C.
Case, Lucius W.
Culbertson, Clyde G.
Dobos, Emeric I.
Edgar, Jas. D.
Elton, Norman W.
Evans, Newton
Goforth, John L.
Gordon, Harold
Heck, F. J.
Hellwig, Christian
Howard, Stacy C.
Kemp, Hardy A.
Kitzmiller, Karl Vivian
Konzelmann, Frank W.
Kosky, Alfred A.
Leichliter, John W.
Lewis, Seaborn J.
Norris, Jack C.
Oesterlin, Ernest J.
Parker, Marian E.
Pessin, Samuel B.
Pons, Carlos A.
Reinhart, Harry L.
Roth, Joseph F.
Seeoof, David Philip
Shafer, Rudolph J.
Shirey, Ralph W.
Stout, Sidney E.
Summerlin, Harold S.
Thorsness, Edwin T.
Tripoli, Carlo J.
Wallace, Stuart A.
Weeter, Harry M.
Wenner, Thos. J.
Williford, Herman B.
Wright, Arthur Wm.
Young, Anna May

T. B. MAGATH.

BOOK REVIEWS

Practical Hematological Diagnosis. By O. H. Perry Pepper and David L. Farley. Pp. 562, 1933. London, Philadelphia, W. B. Saunders Company, $6.00.

This book was primarily designed to lessen the confusion which has arisen due to the numerous terms and methods employed in clinical hematology. The authors desired to present the practical aspects of hematology in simple terms. To this end they have succeeded very well although it is evident that the subject is entirely too complicated to ever hope to make a very simple discussion.

The book is divided into three parts, the first of which covers the components of the blood, the methods for their study and the practical significance of the results obtained. After discussing the cellular elements with little or no reference to the serum and plasma, chapters are devoted to coagulation of blood, blood groups and parasites of the blood.

The second part deals with hematologic findings in the diseases and disorders of the hematopoietic system with chapters on the anemias, polycythemia, the leukemias, purpuric and hemorrhagic diseases, the hematology of infancy and the effects of irradiation and splenectomy.

The last part is concerned with the hematologic findings of diseases not primarily of the blood and consists of an alphabetical list of essential blood pictures in a large list of diseases. The closing chapter is a glossary of hematologic terminology. Throughout the text numerous references appear covering the major recent contributions to the subject.

The general and specific discussions are brief and clear and will appeal primarily to the clinician. Methods were selected arbitrarily although in some instances as for example hemoglobin, numerous methods are given. In this respect the authors have not made the book so practical. Although a half page is devoted to the use of buffers in staining, the formula for the buffer is not given. No method is given for reticulocyte staining and Wright's staining technic is the only one to which reference is made. No

mention of the preparation of blood smears on slides can be found, the coverglass method, which is practical only in the hands of experts, is the only one indicated. All the recent elaborate work on the errors of differential counting is omitted. The prothrombin time test, given in detail, is without reference to Howell's own criticism of his original paper and blood volume is too briefly mentioned for the reader to appreciate any of the problems involved.

While these may or may not be valid criticisms of the text, the book will be useful to clinicians who cannot possibly delve into the intricacies of the subject in the way that clinical pathologists and specialists in the field have found it necessary.

Lymphatics, Lymph and Tissue Fluid. By CECIL K. DRINKER AND MADELEINE E. FIELD. Pp. vii + 254, 1933. Baltimore, The Williams & Wilkins Company. $3.00.

Important as the lymphatic system is there have been very few comprehensive monographs written on the subject. Physiologists, anatomists, pathologists and internists will therefore be delighted to have at hand a modern treatise, brief yet comprehensive, dealing with this difficult subject. The monograph has come as the result of seven years intensive study of the lymphatics and lymph and deals with the structural basis of the lymphatic system, the entrance of foreign particles and colloidal solutions into lymphatics, the permeability of the blood capillaries, the limits of the permeability of the typical normal blood capillary to colloids, the flow of lymph, the composition of lymph, the tissue fluid and finally practical considerations involving a host of clinical questions. The style of the book is excellent and extremely clear, the reader at no time being in doubt as to the status of a particular problem at the present time. This feature is made very clear by an unusual author and bibliography index. From this one can tell at once where to find any reference to previous work in the text. The authors have interpolated numerous tables which briefly and completely describe the various components of the lymph and the results of numerous experiments such as the effect of drugs, salts, extracts, position of the animal, blood pressure, etc.

This is surely an important monograph and must be available to serious students of medical subjects.